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ORGANISMS OF B. LACTIMORBI GROUP FOUND IN THROAT CULTURES AND SIMULATING B. DIPHTHERIAE

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Some years ago, before the routine of tests for virulence had been established, complaints were received that several patients were being quarantined for diphtheria for unusually long periods. Examination of the eighteen-hour serum cultures from the nose and throat showed a rod form with bipolar metachromatic granules, and with marked tendency to parallel arrangement. The organism was isolated, and it was at once clear that we were dealing with a form which did not belong even to the diphtheria group, as it was motile in the hanging drop and showed terminal spores in older cultures. The patients were discharged from quarantine and a brief note was presented to the Association of Pathologists and Bacteriologists in 1907 or 1908. It was our intention to carry out detailed investigation, but the cultures were lost and for a time no more such organisms appeared. In 1910 they were again noticed, but the morphologic distinctions were understood by the diagnostician and the patients were not isolated. For various reasons no further work was done other than to note the constant recurrence of the organism in cultures from various parts of the town, but the frequency of this recurrence, and the fact that the organism so closely resembles diphtheria and has not hitherto been described in the throat, makes a brief report valuable.

In the meantime the work of Jordan and Harris[1] had appeared, and their description of an organism found in association with milk sickness, and named by them B. lactimorbi, agreed closely with my own earlier findings. Later work by these investigators showed that the organism was probably from soil and had no relation to the disease, but the name is now established.

A detailed description on the official blanks of the Association of American Bacteriologists has been made, but the work will not be taken up in detail further than concerns the essential points, together with the group number.

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[1] Jour. Infect. Dis., 1909, 6, p. 401.

GROUP NUMBER—211.3333533

There are evidently several strains, which differ mainly in degree, some having a tendency to long forms and some to short forms, some showing earlier spore formation than others, and so forth, but so far none have been found which did not conform to the group number as noted above.

Morphology.—There are rods with about the same size and the same variations as the C type of diphtheria in Wesbrook's classification and containing deeply stained metachromatic granules. These granules are usually bipolar but may be unipolar, or even central. The granules are usually of the same diameter as the cell, and do not cause the apparent bulging usually noted in diphtheria. This is so characteristic that as soon as one becomes accustomed to the organism, it can readily be

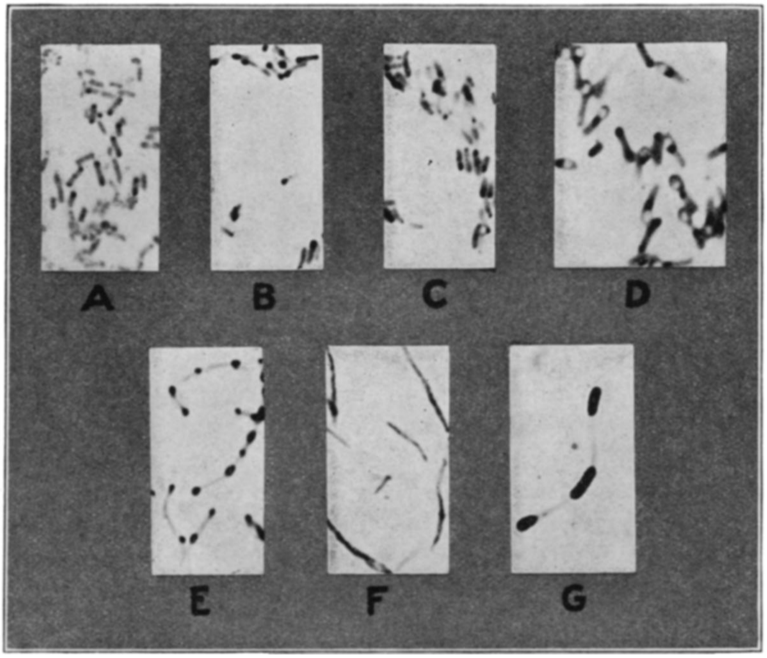

Fig. 1.—A, pure culture, 1911, × 1500, 18 hours, blood serum; B, pure culture, 1921, × 1500, 18 hours, blood serum; C, throat culture, 1921, × 1500, 18 hours, blood serum; D, spores, 1921, × 2000, 30 hours, glycerine agar; E, involution (?), 1921, × 1500, 48 hours, glycerine agar; F, involution, 1921, × 1500, 20 days, gelatin: G. involution, 1921, × 1500, 30 days, milk. With the exception of A, all were stained with methylene blue (Loeffler's), diluted 1: 50 with water, for from 4 to 10 seconds. Photographed by the method described by Charles F. Brush in The Physical Review, 1910, 31; average exposure, 20 seconds on panchromatic plate, development 9 minutes.

distinguished in the routine throat cultures. When it is present in association with true diphtheria, differentiation is more difficult. The intergranular space stains lightly, and with special stains such as Albert's, both body and granules react in a manner identical with diphtheria.

There is a marked tendency to parallel arrangement, though perhaps the average number per bundle is less than with diphtheria.

On favorable mediums such as fresh blood serum, there are frequently no spores in the first 24 hours, while on dried out tubes these naturally appear earlier. The spores are almost always terminal, much like those in tetanus, but somewhat more oval, and about double the diameter of the cell. There is usually a terminal granule at the base of the spore, but occasionally the spore develops slightly more centrally, pushing the granule ahead of it to the extreme tip. The spores stain readily by the usual methods.

The metachromatic granules develop early, and in all mediums except in broth, in which few granules are found, without any preliminary solid stage, so that four- or five-hour cultures have about the same morphology as those of from 15 to 20 hours. In old cultures, especially in gelatin and in milk, there are found mostly involution forms, with great irregularity of the staining material, and of the morphology, and strangely enough, scarcely any spores are seen. These cultures are viable, and the new generation conforms to type.

In the hanging drop there is active motility, and peritrichous flagella have been demonstrated. From the water of condensation of agar or from broth there is a marked tendency to long chains which move as a unit in undulating lines, the granules clearly visible on account of the difference in refractive index. Incidentally, it has been found that by the use of very dilute stains, a small amount of impregnation of the granules can be obtained before cessation of motility, but these faintly stained organisms are no longer viable and soon die if let alone. Gram's stain is negative, a further distinction from the Klebs-Loeffler bacillus.

The organisms are not acid-fast.

Cultural Characteristics.—The growth on the surface of solid mediums is not characteristic, resembling in a general way that of the colon group. On the chart the growth on agar is described as abundant, spreading, flat, glistening, smooth, translucent, butyrous, and yellowish in old cultures, but without notable odor. On blood serum it is about the same but usually rather more raised, probably because the medium is less fresh than the routine agar.

In broth there is a membranous pellicle, easily disturbed and sinking to the bottom, with a moderate cloud and an abundant sediment, viscid on shaking. In old cultures the broth usually becomes almost clear with a ball-like mass at the bottom, composed of the long involution forms noted in the foregoing. There is no odor and no pigment formation.

In gelatin the growth at room temperature is very slow, with a gradual funnel liquefaction, which tends to evaporate, leaving a cup-like depression. In gelatin at 37 C. there is complete loss of power to resolidify at the end of 48 hours, and the subsequent appearance of the culture is much like that of old broth.

Chemically the organism is inert. There is no fermentation of sugars, no diastatic action, no nitrate reduction. In milk there is a continuous alkaline reaction with peptonization, beginning about the seventh day at 37 C. and complete at about the tenth day. Blood serum is slowly liquefied, best seen when kept in a moist atmosphere to prevent drying. Luckhardt[2] found virulence in one strain, but was unable to find it in others, and so far none has been noted for small animals in this present series. No experiments in feeding have been made as there are no clinical symptoms connected with the presence of the organism.

Frequency of Appearance.—In the last 8 or 10 years they appear in rather less than 1% of the cases in the Cleveland Health Division Diagnosis Laboratory, and careful controls of mediums and other substances remove the possibility of their being a laboratory contamination. In 1920 they have also been found occasionally in normal throats in class culture work. When present in a throat they persist indefinitely and show no relation to clinical phenomena. They appear to be occasional saprophytic inhabitants of the upper air passages, and when their hosts become infected with diphtheria there is a period in which both species are present. The diphtheria bacillus then disappears, but the other persists, and unless the morphology is recognized, there is a distinct probability that the release cultures will continue to be reported as positive, until virulence tests are made.

We have also found the organism of great value in teaching, especially in the elementary work, in which the combination in one organism of motility, spores, metachromatic granules and harmlessness, is a great trouble saver.

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[2] Jour. Infect. Dis., 1909, 6, p. 492.

The accompanying illustration shows the morphology alone and in mixed cultures, and requires no special explanation.

SUMMARY

In normal and pathologic throats organisms occur from time to time which under routine conditions so closely resemble B. diphtheriae as to cause possible confusion.

These organisms may be readily differentiated, even under these conditions, by certain constant morphologic characteristics, and when isolated spores and motility are seen.